NEW DRUG DELIVERY SYSTEM OF DICLOFENAC SODIUM FOR ITS APPLICATION IN THERAPEUTIC EMBOLIZATION STRATEGY.

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الخلاصة.

ان الشكل الدائري المثالي للكبسولات المصغرة لمادة الراتنج المتبادل الايون المسمى داي اثيل امينو اثيل –ترايز – أ كريل (DEAE) قد استخدمت مسبقا بنجاح لتحقيق غلق ميكانيكي للاوعية الدموية ولاول مرة ومن خلال هذا البحث تم أمدصاص مادة الدكلوفيناك صوديوم على كبسولات DEAE وبنسبة 28.3 % واظهرت دراسة الذوبان أن تحرر الدواء كان سريعا من هذه الكبسولات المحملة وهذا قد يؤدي الى نقص سريع بالجرعة الدوائية قبل وصول هذه الكبسولات المحملة بالدواء الى جهة الهدف ولذلك تم أيضا في هذه الدراسة تغليف هذه الكبسولات المحملة بالدواء بواسطة البولمر PLGA وباستخدام طريقتين مختلفتين للتغليف واظهرت النتائج ان زيادة نسبة البولمر المستخدم في التغليف يؤدي الى تباطؤ اكثر في تحررالدواء من خلال تاثيره على نسبة المحور الى الغلاف وكذلك تأثيره على ميكانيكية التحرر. وقد بينت الدراسة ان استخدام PLGA 60 % للتغليف بواسطة طريقة المستحلب تعطي كبسولات مصغرة ذات شكل دائري منتظم وتحرر بطيء منتظم للدواء وهذا مما يجعل هذه الصيغة التركيبية يمكن ان تحضر على شكل محلول زرق لكي يزرق بشكل مباشر في المكان المطلوب وبذلك يقدم البحث نظام نقل دوائي جديد لماده الدكلوفيناك وكذلك استخدام جديد للدواء في الستراتيجية الجديدة الواعدة باستخدام جسيمات لها مفعول مضاد للالتهابات لعمل انسدادات علاجية في الاورده.

Abstract

The perfectly spherical microspheres of the ion- exchange resins diethylaminoethyl – trisacryl (DEAE) had been successfully used for achieving mechanical occlusion of vascular lumen. In this study; diclofenac sodium is reversibly adsorbed onto DEAE microspheres (28.3 g drug / 100 g of microspheres). The dissolution profile showed rapid release of the drug from the loaded microspheres, and since this may lead to dramatic decrease in the drug before the microspheres. reaches the targeted site, therefore, microencapsulation of the drug loaded DEAE microspheres is carried out for the first time in this study using PLGA as a coating polymer and applying two different methods of microencapsulation. The results showed that increasing the amount of PLGA lead to more retardation in the release of the drug through affecting core : coat ratio and the mechanism of drug release. It is also found that applying emulsification / solvent

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evaporation method with 60% w/w PLGA gave regular retarded release microcapsules with better sphericity, which may be easily injected by a catheter at the required site and offers a new drug delivery system and new application for diclofenac sodium in the promising strategy of using embolization particles with anti – inflammatory activity.

Introduction

Ion exchange resins have occupied an important place in the development of novel drug delivery system for their better drug – retaining properties, prevention of dose dumping and more uniform release of drug than simple matrices (1,2,3). Microspheres of the resins have been used with success for mechanical occlusion of vascular lumen (embolization) to treat different types of tumors in different organs (4,5). The efficacy of therapeutic embolization is based on keeping the embolic region away from blood circulation, however, inflammation caused by the occluding materials had been identified as a major enhancer of revascularization of the occluded vascular lumen (5). Therefore, embolization particles provided anti- lammatory activity should be a promising strategy (6). Microencapslation of the ion exchange resin microspheres containing the drug provides better control release due to the presence of rate- controlling membrane than the uncoated microspheres (3). Diethylaminoethyl (DEAE) trisacryl LS is the ion- exchange resin microspheres used in this study for their specific characteristic properties in embolization therapy (7,8) and diclofenac sodium is selected for the nature of its ionizable charge and for its potential effective action as a non-steroidal anti- inflammatory drug widely used in the long term therapy of chronic inflammatory conditions, and prevention of post-embolization revascularization (9,10). The aim of this study is to successed in loading diclofenac sodium on the ion- exchanger (DEAE), and studying its release profile, in addition to modifying the release through coating the drug loaded microspheres with PLGA applying different methods of encapsulation. This work may offer a new drug delivery system for diclofenac sodium (to be injected by a catheter) and new application of the drug in the promising strategy of using embolization particles with anti-inflammatory activity.

Materials and Methods

Materials

The following chemicals were used as received:

Dichloromethan (Merck, Darmstad, Germany), silicon oil (Dow croning 556, cosmetic grade, Seneffe, Belgium), Diethylaminoethyl (DEAE) Trisacryl LS microspheres (BioSepra s.a., France), Poly (d,l – lactide – co- glycolide) PLGA (50:50, m.wt. 56,500, Boehringer, Ingelheim, Germany), diclofenac sodium (m.wt. 318.1), n- hexane and methanol (Fisher Scientific UK Limited, U.K.).

Methods

1-Loading of DEAE microspheres with the drug :-
1% w/v of diclofenac sodium solution was prepared by dissolving 1 gm of the drug in 100 ml of distilled water, 3g of DEAE Trisacrly LS microspheres powder of 80 –

160 nm diameter with a mean diameter of 120 nm was added and magnetically stirred for 60 minutes, filtered by Millipore filter (41 µm) (6) , and the absorbance of the filtrate was measured by U.V. at 275 nm for diclofenac sodium.

2-Microencapsulation of DEAE microspheres containing diclofenac sodium:-
Two methods were used to prepare microcapsules.

A-Coacervation phase separation method:-
50 mg of the microspheres containing drug were dispersed in solutions containing 10%, 25% ,30% and 50% w/w PLGA dissolved in dichloromethan. The dispersion was stirred at 200 r.p.m. at room temperature and silicon oil was added with flow rate 0.3 ml/ min to the above dispersions with continuous stirring until phase separation occured. The prepared microparticles were filtered, dispersed in n-hexane and dried. Then washed with distilled water, filtered using millipore filter (41 µm), congealed and lyophilized (11,12).

B-Emulsification / solvent evaporation method :
In this method two phases were prepared; the organic and aqueous phases; The organic phase composed of solutions containing 20%, 50%,60% and 80% w/w PLGA dissolved in dichloromethan. To each one of above solutions 50 mg of the microspheres containing drug was added. The aqueous phase composed of mixture of 200 ml water, 1.5 ml polyvinyl alcohol and 10 ml methanol. The two phases were mixed together and evaporated under reduced pressure until microcapsules were obtained. These microcapsules dispersed in n- hexane and dried. Then washed with distilled water , filtered using millipore filter (41 µm) and lyophilized (13).

3-Microscopical evaluation :
The morphology of the prepared microcapsules was examined by optical microscope (Nikon microscope, Nikon Corporation Instruments Division, Yokohama Plant).

4-In vitro release of the drug :
The release of diclofenac sodium from the drug loaded DEAE microspheres and the microcapsules (which are prepared by coating the drug loaded microspheres with different percentages of PLGA), was determined in saline buffer solution at 37C and 50 r.p.m. using dissolution tester with UV/VIS spectrophotometer lambda 40 (Perkin-Elmer spectrophotometer). The release was followed in the salin buffer pH 7.4 at 275 nm U.V light (11).

Result and Discussion

Microspheres of diethyl amino ethyl trisacryl LS (DEAE microspheres) are powder of 80-160 nm diameter with a mean diameter of 120 nm. DEAE is ion- exchanger of cationic acrylic derivatives. The ionizable groups are mixture of weak tertiary amines and strong quaternary amines (6). Diclofenac has negative charge ,therefore, it is adsorbed onto DEAE microspheres. In this study it is found that 0.65 g of the added drug is adsorbed on 3 g of DEAE microspheres (28.3% w/w) means that each 100g of the microspheres containing 28.3 g diclofenac sodium and this is calculated by measuring the amount of the drug remained un adsorbed in the filtrate using UV spectrophotometer according to batch technology (6). The results also showed that 65.7 % w/w of the total amount of the drug is used in the adsorption process, and this indicates that some of the charged group on DEAE are not available for exchange with the drug due to steric hindrance effect. It

can be concluded that the DEAE are the most suitable microspheres for binding of diclofenac sodium as it exhibits a high binding capacity. Studying the release of diclofenac sodium from DEAE microspheres containing drug showed very rapid release (fig .1) (within 1-5 minutes) , and such a release profile may lead to dramatic release of the drug before the microspheres reach the targeted site. Therefore, microencapsulation (coating) of the DEAE microspheres containing drug was carried out in this study using PLGA as the coating polymer since it is biocompatible and biodegradable polymer (15,16), and it had been widely used as release controlling material (17,18).

Two methods were applied for microencapsulation in this study; coacervation / phase separation and emulsification / solvent evaporation method. In the first method, the DEAE microspheres containing drug were coated with 10%,25%,30% and 50% PLGA, the efficiency of microencapsulation was very low at PLGA ratio higher than 50%., and this could be attributed to a mismatching of the water-coacervate and water-supernatant interfacial tension (11,19) . In the emulsification /solvent evaporation method, it was possible to use higher percentages of the coating material (20%,50%,60% and 80% w/w) since there is no phase separation (13) and it gave high encapsulation efficiency.

Fig. (2) shows the shape of DEAE microspheres alone, in addition to microcapsules of DEAE microspheres containing drug prepared by the two microencapsulation methods, and it appears that DEAE microspheres. are round and spherical in shape while coated microspheres showed thickening on the surface due to drug adsorption. The microcapsules of the drug loaded microspheres prepared by emulsification / solvent evaporation method were spherical in shape, reproducible and well separated with an average diameter of 57 µm while microcapsules prepared by coacervation method, their shape and the outer surface were not easily to be controlled with diameter of 71 µm because the process of microencapsulation is frequently impaired by the residual solvent and coacervating agent (11). Therefore, this study recommended to use the emulsification method for microencapsulation of DEAE microspheres containing drug since it is well suited for producing microcapsules in low size rang and better sphericity which are important requirements for dosage form to be injected through microcatheter. The comparative study of the release of diclofenac sodium from the coated drug loaded microspheres prepared by the two methods (Fig. 3 and 4) showed that the release of the drug decreased as the amount of PLGA increased since it affects core : coat ratio (20) as well as the mechanism of drug release where erosion could be the major mechanism for microcapsules with low percentages of PLGA while diffusion might be the main mechanism for the slow release of drug from microcapsules with high percentages of PLGA using both methods (21,22). However, it was found in this study that microcapsules with 60% w/w PLGA prepared by emulsification / solvent evaporation method gave more regular retarted release and better spherical shape with low diameter (57 µm), therefore it is suggested to be used for formulation of injectable dosage form. As a conclusion, ion-exchange resin DEAE microspheres can be used successfully to prepare a new drug delivery system for diclofenac sodium with high efficiency and fast release.Microencapsulation of the drug loaded DEAE microspheres with PLGA as a coating polymer retarded the release of the drug and offered a new drug delivery system that can be injected by a catheter to induce embolization as well as reduces the accompanied pain and revascularization.

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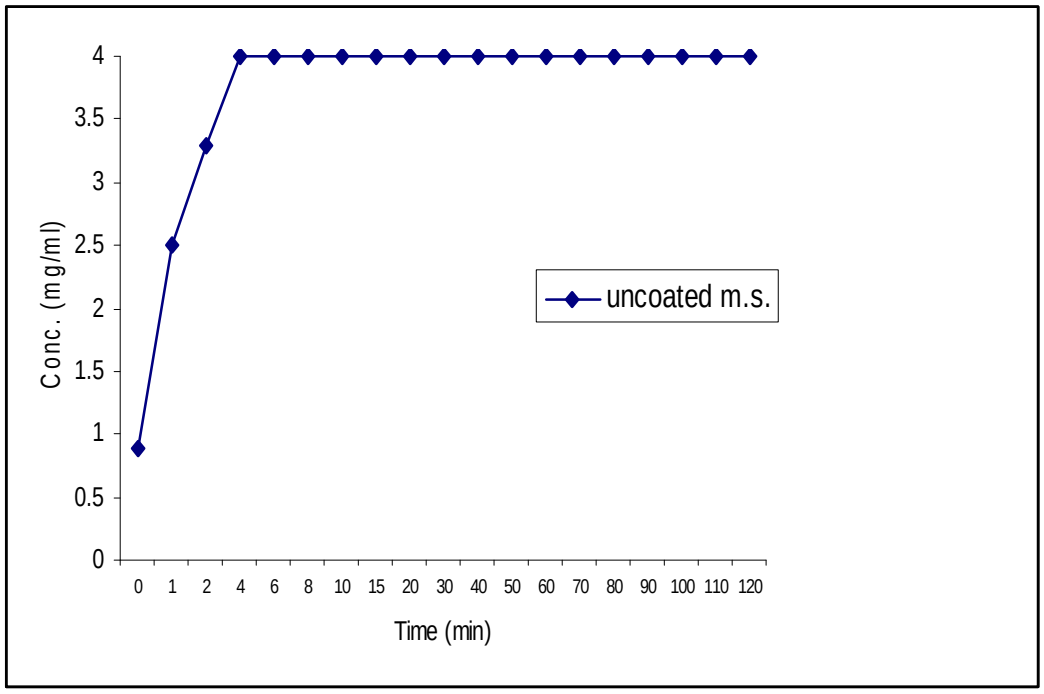
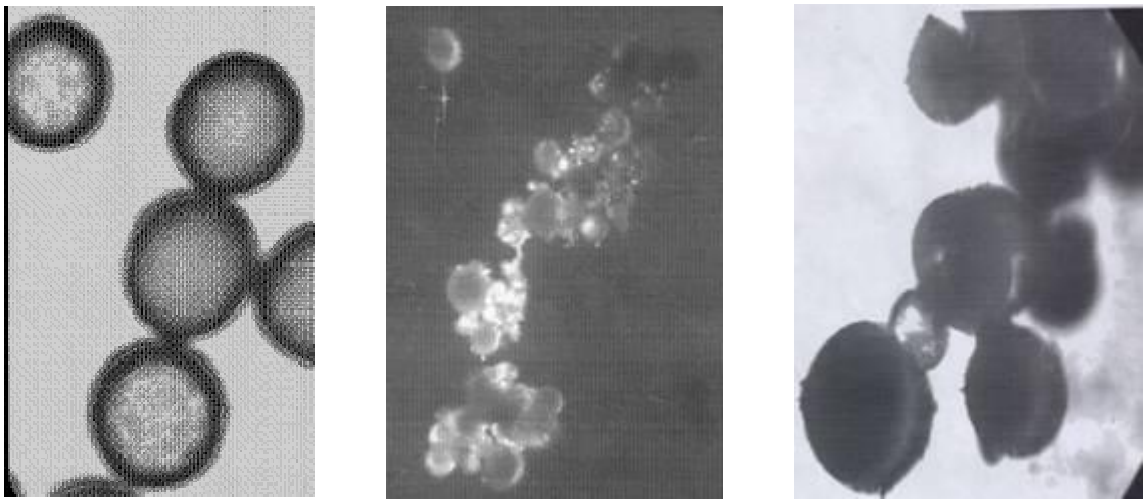


Fig. 1. Release of diclofenac sodium from uncoated drug loaded microspheres.



A **B** **C**

Fig. 2. Microscopical evaluation. A. Unloaded (DEAE) microspheres, B. Microcapsules of drug loaded microspheres (by coacervation method), C. Microcapsules of drug loaded microspheres (by emulsification method).

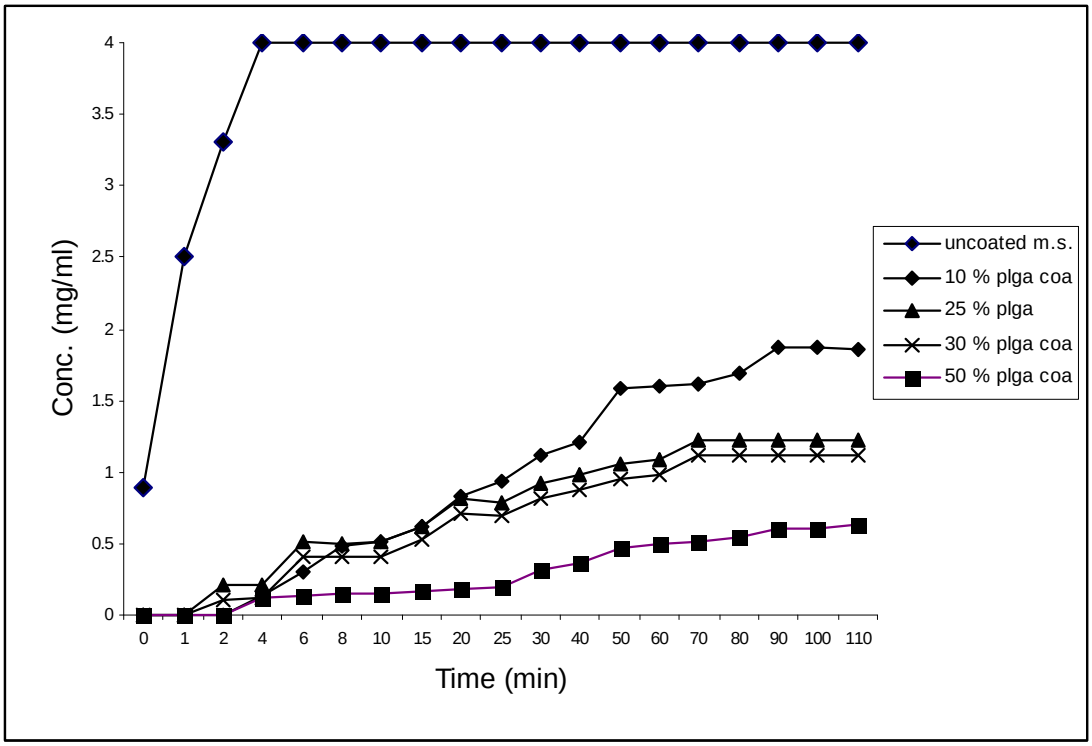


Fig. 3. Release of diclofenac sodium from microcapsules of drug loaded microspheres prepared by coacervation method.

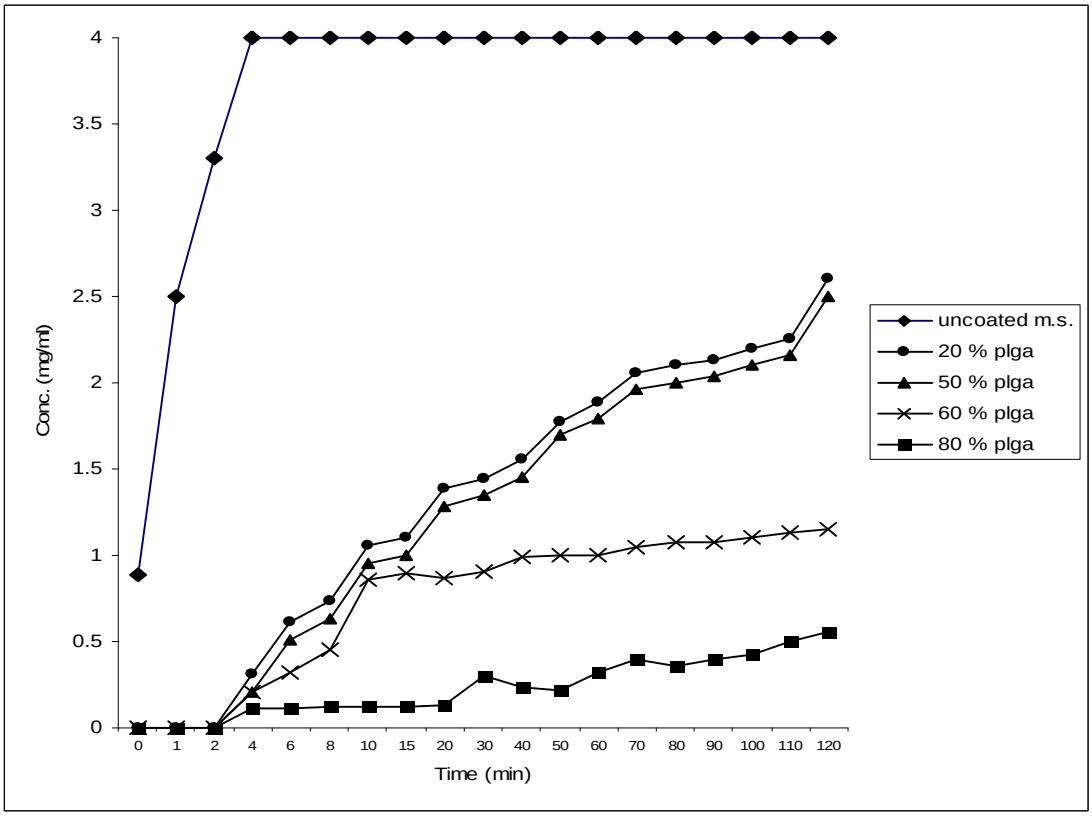


Fig.4. Release of diclofenac sodium from microcapsules of drug loaded microspheres prepared by emulsification / solvent evaporation method.